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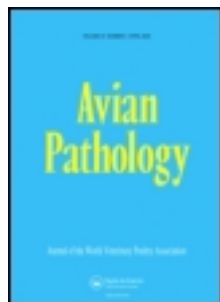
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## HAEMATOLOGICAL CHANGES ASSOCIATED WITH FOOD-RELATED ORAL LESIONS IN BROWN LEGHORN HENS

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### SUMMARY

After 19 weeks on a mash diet nine of the 10 birds exhibited from two to 19 oral lesions. The majority of these lesions involved the total erosion of the buccal epidermis giving a total area of exposed dermis of 0.6 to 94.5 mm<sup>2</sup>. This was accompanied by a significant reduction in the numbers of heterophils, monocytes and eosinophils. Birds with lesions also had significantly reduced concentrations of haemoglobin and may have been suffering from a mild form of microcytic normochromic anaemia without the complication of an extensive bone marrow involvement. These findings have implications for poultry welfare.

### INTRODUCTION

Extensive chronic erosion of the buccal epithelium has been reported in adult hens fed on a mash diet (Gentle, 1986). This erosion took the form of a discrete ulcerated lesion infected with streptococci, staphylococci and in some cases *Pasteurella multocida*. During the development of these lesions the epithelium remained intact but showed considerable thinning with an overlaying plug of tissue debris. In the plug were numerous bacterial colonies and large numbers of heterophils were present in the epithelial and subepithelial tissues. In more advanced cases the epithelium was completely eroded with dermis exposed. The dermis adjacent to the necrotic tissue plug showed lakes of oedema, large numbers of heterophils, reactive fibroblasts and proliferating capillaries. Birds with oral lesions continued to feed normally but when substances known to be painful in the human blister-base test were introduced into the buccal cavity they behaved in a manner similar to that during thermal nociceptive stimulation (Woolley and Gentle, 1987; Gentle and Hill, 1987). Birds with oral lesions may therefore be in discomfort and are likely to be stressed. A number of physiological methods,

especially the adrenal response, have been used to measure stress (Freeman, 1985) but changes in peripheral blood leucocytes (Newcomer, 1958; Wolford and Ringer, 1962; Ben Nathan *et al.*, 1976) and the heterophil/lymphocyte ratio are regarded as especially good measures of the fowl's perception of environmental stress (Gross and Siegel, 1983). An earlier report (Gentle 1986) was concerned with the aetiology of food-related oral lesions and the present study was designed to investigate the haematological profile of the birds to assess if the pathology could be correlated with any sub-clinical changes.

## MATERIALS AND METHODS

### *Experimental procedure*

Thirty adult Brown Leghorn hens (30 weeks of age) were used which had been bred and reared at the Institute of Animal Physiology and Genetics Research. They were housed in individual battery cages and allowed access to food and water *ad libitum*. Before the start of the experiment all birds had been fed on a pelleted diet and had normal healthy buccal epithelia. Fifteen birds were then transferred to the same diet in a mash form and the remaining birds continued to be fed the pelleted diet. Blood samples were removed from the brachial vein in all birds after 9, 14 and 19 weeks, the blood being dispelled immediately from the sterile disposable plastic syringes into collecting tubes containing sequestrene (dipotassium salt of ethylene tetra-acetic acid-EDTA, 1.5 mg/ml of blood) anticoagulant. After 14 weeks five birds fed on the mash diet (experimental birds) and four birds fed on the pelleted diet (control birds) were killed and tissue samples taken and at the end of the experiment (19 weeks) all birds were killed with intravenous sodium pentobarbitone (Sagatal, May & Baker Ltd) weighed and samples taken.

### *Blood tests*

The haematological tests included: haemoglobin (Hb) measurements; packed cell volume (PCV); red blood cell (RBC), white blood cell (WBC) and thrombocyte (Thr) counts and calculations of the indices; mean cell haemoglobin (MCH); mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) (Maxwell, 1981). The counts were recorded using an electronic particle counter (Industrial D Model, Coulter Electronics Ltd., Luton). The following parameters were judged to give good 'plateaux' for fowl blood cells: orifice diameter 100  $\mu\text{m}$ ; attenuation - 1; aperture - 0.017; threshold - 10. The counter was calibrated with cell control 4C (Coulter Electronics Ltd) and blood from volunteers of laboratory personnel. Reticulocyte counts were performed according to Dacie and Lewis (1975) and the cells were divided into five different stages according to the degree of reticulation (Coates and March, 1966). White cell differential counts were presented as absolute numbers. All counts were carried out by the same investigators and the data were analysed by the Student's *t* test.

### *Collection of tissue samples*

At post mortem specimens of liver, spleen, heart and kidney were removed and placed into 10% neutral buffered formalin for histological examination. Paraffin sections were stained for haemosiderin by Perl's Prussian Blue reaction and by Gram's stain for bacilli.

Bone marrow smears were also made and together with the above stains they were stained by the May-Grunwald and Giemsa method.

Table 1. Mean values of blood cell parameters ( $\pm$  SD)

Parameter	Duration of experiment (weeks)		
	9	14	19
<b>Red blood cells</b>			
Hb (g/100 ml)	9.0 $\pm$ 0.96 (E)	8.1 $\pm$ 0.73	8.8 $\pm$ 1.04*
	9.1 $\pm$ 0.83 (C)	8.3 $\pm$ 0.74	9.9 $\pm$ 1.05
PCV (%)	23.8 $\pm$ 1.74**	24.0 $\pm$ 1.81	23.9 $\pm$ 1.59
	26.0 $\pm$ 2.80	24.7 $\pm$ 2.09	25.4 $\pm$ 2.36
RBC ( $10^6$ /mm <sup>3</sup> )	1.88 $\pm$ 0.20	2.28 $\pm$ 0.20	1.94 $\pm$ 0.18
	1.85 $\pm$ 0.26	1.98 $\pm$ 0.28	2.04 $\pm$ 0.21
MCH (pg)	47.8 $\pm$ 3.78	36.0 $\pm$ 3.12***	46.0 $\pm$ 4.73
	50.4 $\pm$ 9.16	42.8 $\pm$ 5.47	48.8 $\pm$ 6.05
MCHC (%)	37.8 $\pm$ 2.92	33.8 $\pm$ 1.98	37.1 $\pm$ 2.75
	35.5 $\pm$ 4.72	33.9 $\pm$ 2.06	39.1 $\pm$ 3.27
MCV ( $\mu$ m <sup>3</sup> )	126.9 $\pm$ 11.93**	105.5 $\pm$ 4.58***	123.9 $\pm$ 7.14
	141.7 $\pm$ 16.32	126.2 $\pm$ 12.91	125.1 $\pm$ 11.89
Reticulocytes (%)	—	11.3 $\pm$ 1.30	9.6 $\pm$ 1.84
	—	12.1 $\pm$ 2.54	9.9 $\pm$ 2.31
<b>White blood cells</b>			
WBC ( $10^3$ /mm <sup>3</sup> )	14,211 $\pm$ 7,515 (E)	19,483 $\pm$ 5,892	12,325 $\pm$ 2,909
	18,296 $\pm$ 6,350 (C)	15,834 $\pm$ 4,488	15,771 $\pm$ 5,264
Heterophils (H) ( $10^3$ /mm <sup>3</sup> )	5,003 $\pm$ 6,091	5,068 $\pm$ 2,365	3,020 $\pm$ 1,150*
	3,902 $\pm$ 2,925	3,718 $\pm$ 2,139	4,667 $\pm$ 1,967
Eosinophils ( $10^3$ /mm <sup>3</sup> )	209 $\pm$ 172	333 $\pm$ 283	100 $\pm$ 100**
	175 $\pm$ 289	284 $\pm$ 326	372 $\pm$ 255
Basophils ( $10^3$ /mm <sup>3</sup> )	121 $\pm$ 100	211 $\pm$ 218	313 $\pm$ 214
	229 $\pm$ 228	283 $\pm$ 248	330 $\pm$ 341
Lymphocytes (L) ( $10^3$ /mm <sup>3</sup> )	7,957 $\pm$ 2,812**	12,829 $\pm$ 4,012	8,518 $\pm$ 2,153
	12,811 $\pm$ 5,010	10,728 $\pm$ 3,122	9,123 $\pm$ 2,991
Monocytes ( $10^3$ /mm <sup>3</sup> )	921 $\pm$ 530	1,043 $\pm$ 728	406 $\pm$ 258*
	1,178 $\pm$ 801	821 $\pm$ 606	1,282 $\pm$ 1,043
Thrombocytes ( $10^3$ /mm <sup>3</sup> )	34,086 $\pm$ 8,565	42,288 $\pm$ 12,059	38,535 $\pm$ 10,227
	31,125 $\pm$ 6,992	48,203 $\pm$ 21,375	44,496 $\pm$ 8,588
H/L Ratio	0.62 $\pm$ 0.68	0.42 $\pm$ 0.22	0.36 $\pm$ 0.13
	0.34 $\pm$ 0.27	0.36 $\pm$ 0.18	0.55 $\pm$ 0.27

E = Experimental C = Control.

\*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.02$ ; \* =  $P < 0.05$ .

The visible oral lesions were excised from the mouth with surrounding normal tissue and fixed in formal-acetic-alcohol. The fixed tissue samples were embedded in paraffin wax, cut vertically at 8  $\mu\text{m}$ , and stained with haematoxylin and eosin (H&E). The size of each lesion was measured from the section and they were graded in severity from 1 to 4: grade 1, superficial covering of necrotic tissue on the buccal epithelium with little if any erosion; grade 2, clear erosion of the epithelium with a loss of up to 50% of its thickness; grade 3, extensive epithelial erosion with a loss of over 50% of its thickness but with epithelium still present; grade 4, complete loss of buccal epithelium with extensive damage to underlying dermis.

## RESULTS

### *Haematological tests*

The results are shown in Table 1. After being on a mash diet for 19 weeks the birds showed a reduction in haemoglobin ( $P<0.05$ ). The packed cell volume and number of red cells also declined in the experimental birds but not significantly. The mean cell volume and mean cell haemoglobin were both significantly reduced ( $P<0.001$ ) after the birds had been fed a mash for 14 weeks. Neither the mean cell haemoglobin concentrations nor the reticulocyte levels showed any prominent differences.

After 9 weeks the birds fed on the mash diet were showing a decrease in lymphocytes ( $P<0.01$ ) and a slight but non-significant increase in heterophils which also gave an elevated but non-significant increase in H/L ratio. By the 19 weeks on the mash diet there were reductions in the numbers of heterophils ( $P<0.05$ ), monocytes ( $P<0.05$ ) and eosinophils ( $P<0.01$ ).

### *Tissue examination*

None of the sections from the experimental birds stained by Perl's Prussian Blue reaction or Gram's stain revealed haemosiderin or Gram positive bacilli. The bone marrow smears showed no haemosiderin but some appeared hyperplastic.

An example of extensive oral lesions is shown in Fig. 1 taken from a bird killed at 14 weeks. After 19 weeks nine of the 10 birds fed on the mash diet exhibited oral lesions which ranged in number from 2 to 19 (mean  $7.7 \pm 2$ ) in the oral cavity. The majority of these lesions fell into the most severe category and the total lesion area in this grade varied from 0.6 to 94.5  $\text{mm}^2$  (mean  $37.7 \pm 9 \text{ mm}^2$ ). Four of the birds fed on the pelleted diet also showed lesions but none more than two small lesions. Three of the birds fed on the mash diet also showed extensive bacterial damage to the salivary glands which has not been previously observed. It is not known what effect this would have on the production of saliva but from the number of salivary glands involved and the extent of tissue damage salivary flow was likely to be affected. There were no significant correlation between the number of lesions or total lesion areas and the haematological measurement taken. Experimental birds weighed less than their controls (1293 g vs 1511 g;  $P<0.05$ ).

## DISCUSSION

The significant changes in the haematological profile of the experimental birds fed on the mash diet showed reduced mean cell haemoglobin and accompanying mean cell volume at 14 weeks and reductions in haemoglobin, heterophila,



*Fig. 1. Photograph of the mouth of a bird showing oral lesions. The right side of the mouth was cut so that the whole of the mouth is visible and the tongue has been pulled to one side. The lesions in the lower buccal epithelium can be clearly seen as well as the single palatal lesion (lesions arrowed). Food can also be seen clinging to the mouth, which is typical of mash-fed birds.*



eosinophils and monocyte numbers at 19 weeks.

Decreases in the value of red blood cell parameters were observed in young chickens following feeding or injection of phenylhydrazine (Stino and Washburn, 1970a,b), a powerful haemolytic agent which when given to chickens, produced chronic anaemic stress (Stino and Washburn, 1970b). The comparisons between this experimentally-induced anaemia and the condition produced in the present study in respect to the red cell parameters were similar with common reductions in Hb, PCV, RBCs and MCHC. The MCV values in this study were significantly reduced at 9 weeks but by the end of the experiment they had recovered to show values similar to the control birds. This recovery may have been due to an increase in bone marrow activity resulting in an increase in MCV (Stino and Washburn, 1970b) and some hyperplasia was seen in the present study.

There were some interesting contradictory findings with the different white blood cells. The mean number of heterophils and the heterophil/lymphocyte ratios were not significantly increased as a result of the oral lesions which would indicate that the birds may not have been unduly stressed by the treatment or the resulting lesions (Gross and Siegel, 1983). Eosinophils were, however, significantly reduced in number in the birds fed on the mash diet suggesting there may have been stress (Maxwell, 1980).

Although a few of the bone marrow samples in the birds fed on the mash diet appeared hyperplastic, the absence of haemosiderin in the tissues examined and in the marrow itself may indicate that the marrow hyperactivity was due to the anaemia. With pelleted food, the individual food intake of birds in the present study was increased. It is possible that differences in food intake may influence certain haematological parameters. For example, when broilers are placed on a restricted diet, there are significant reductions in mean cell volume and mean cell haemoglobin (Maxwell, unpublished data). The absence of infective lesions in viscera would suggest that the haematological changes were a consequence of oral lesions.

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## RESUME

### Modifications hématologiques associées à des lésions orales liées à la nourriture chez des poules Leghorn brunes

Le nombre, l'étendue et la sévérité de lésions orales ulcératives, associées à des modifications hématologiques ont été étudiés chez des poules Leghorn brunes recevant un aliment composé. Après 19 semaines, neuf sur 10 oiseaux ont montré un nombre de lésions orales s'étageant entre deux et 19. La majorité de celles-ci concernait une érosion totale de l'épiderme buccal laissant apparaître le derme sur une surface de 0,6 à 94,5 mm<sup>2</sup>. Ces lésions étaient accompagnées d'une réduction significative du nombre des hétérophiles, monocytes et éosinophiles. Les oiseaux atteints avaient aussi une diminution significative de la concentration en hémoglobine et souffraient d'une forme bénigne d'anémie microcytique normochromique sans complication impliquant massivement la moelle osseuse. Ces résultats ressortissent à un problème de bien-être des animaux.

## ZUSAMMENFASSUNG

### Im Zusammenhang mit oralen, futterbedingten Veränderungen bei braunen Leghornhennen festgestellte veränderte haematologische Parameter

Neun von 10 Tieren zeigten, nachdem sie 19 Wochen ein Mehlfutter erhalten hatten, jeweils zwei bis 19 orale Veränderungen. Bei der Mehrzahl derselben handelte es sich um eine totale Erosion der buccalen Epidermis, was einer insgesamt betroffenen Dermisfläche von 0,6 bis 94,5 mm<sup>2</sup> entsprach. Gleichzeitig bestand eine signifikante Abnahme der Anzahl von Heterophilen, Monozyten und Eosinophilen. Tiere mit Schnabelveränderungen zeigten auch eine signifikant reduzierte Haemoglobinkonzentration und haben vermutlich an einer milden Form der mikrozytären normochromen Anaemie, ohne deutliche Knochenmarksbeteiligung gelitten. Diese Befunde haben sicherlich Einfluß auf das Wohlbefinden der Hühner.

## RESUMEN

### Cambios hematológicos asociados a lesiones orales relacionadas con la ingesta en aves ponedoras Brown Leghorn

Tras 19 semanas de estar alimentados con una dieta de mezclas, nueve de diez aves exhibieron lesiones orales en un número entre 2 y 19. En la mayoría de estas lesiones existía una erosión completa de la epidermis bucal, mostrando un área

total de dermis expuesta de 0.6 a 94.5 mm<sup>2</sup>. Estas lesiones se acompañaron de una evidente reducción en el número de heterófilos, monocitos y eosinófilos. Las aves con lesiones presentaron además una reducción en la concentración de hemoglobina, pudiendo sufrir una forma ligera de anemia normocrómica microcítica sin llegar a existir una extensa afectación de la médula ósea. Dichos hallazgos tienen implicaciones en el bienestar de las aves.